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ADJUVANT THERAPIES USING NORMOBARIC OXYGEN WITH HYPOTHERMIA OR ETHANOL FOR REDUCING HYPERGLYCOLYSIS IN THROMBOEMBOLIC CEREBRAL ISCHEMIA

L. CAI,[a,b†] J. STEVENSON,[b†] C. PENG,[b] R. XIN,[b,c] R. RASTOGI,[b] K. LIU,[b] X. GENG,[a,b] Z. GAO,[d] X. JI,[d] J. A. RAFOLS,[e] Z. JI[a*] AND Y. DING[a,b*]

[a] *China-America Institute of Neuroscience, Department of Neurology, Luhe Hospital, Capital Medical University, Beijing, China*

[b] *Department of Neurological Surgery, Wayne State University School of Medicine, Detroit, MI, USA*

[c] *Department of Radiology, Luhe Hospital, Capital Medical University, Beijing, China*

[d] *Cerebral Vascular Diseases Research Institute, Capital Medical University, Beijing, China*

[e] *Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI, USA*

Abstract—*Background and purpose:* Normobaric oxygen (NBO), ethanol (EtOH), and therapeutic hypothermia (TH) delivered alone or in combination have neuroprotective properties after acute stroke. We used an autologous thromboembolic rat stroke model to assess the additive effects of these treatments for reducing the deleterious effects of hyperglycolysis post-stroke in which reperfusion is induced with recombinant tissue plasminogen activator (rt-PA). *Methods:* Sprague–Dawley rats were subjected to middle cerebral artery (MCA) occlusion with an autologous embolus. One hour after occlusion, rt-PA was administered alone or with NBO (60%), EtOH (1.0 g/kg), TH (33 °C), either singly or in combination. Infarct volume and neurological deficit were assessed at 24 h after rt-PA-induced reperfusion with or without other treatments. The extent of hyperglycolysis, as determined by cerebral glucose and lactate levels was evaluated at 3 and 24 h after rt-PA administration. At the same time points, expressions of glucose transporter 1 (Glut1), glucose transporter 3 (Glut3), phosphofructokinase1 (PFK-1), and lactate dehydrogenase were (LDH) measured by Western blotting. *Results:* Following rt-PA in rats with thromboembolic stroke, NBO combined with TH or EtOH most effectively decreased infarct volume and neurological deficit. As compared to rt-PA alone, EtOH or TH but not NBO monotherapies significantly reduced post-stroke hyperglycolysis. The increased utilization of glucose and production of lactate post-stroke was prevented most effectively when NBO was combined with either EtOH or TH after reperfusion with rt-PA, as shown by the significantly decreased Glut1, Glut3, PFK-1, and LDH levels. *Conclusions:* In a rat thromboembolic stroke model, both EtOH and TH used individually offer neuroprotection after the administration of rt-PA. While NBO monotherapy does not appear to be effective, it significantly potentiates the efficacy of EtOH and TH. The similar neuroprotection and underlying mechanisms pertaining to the attenuation of hyperglycolysis provided by EtOH or TH in combination with NBO suggest a possibility of substituting EtOH for TH. Thus a combination of NBO and EtOH, which are widely available and easily used, could become a novel and effective neuroprotective strategy in the clinical setting. © 2016 Published by Elsevier Ltd. on behalf of IBRO.

Key words: glucose metabolism, ischemia/reperfusion injury, combination therapy, autologous embolus, rt-PA.

INTRODUCTION

The current standard treatment for ischemic stroke is limited to thrombolytics, provided that they are administered within a narrow window of time. Because of such stringent conditions, only 8% of all patients admitted for acute ischemic stroke in the United States end up receiving this treatment (Menon et al., 2015). However, even when thrombolytics are used within the established guidelines, poor clinical outcomes and a high risk of complications often occur (Saver, 2004). Therefore a search for effective alternative or adjuvant therapies that can be effectively translated into clinical practice is warranted.

Following stroke, the restriction of cerebral blood flow and the subsequent unmet demand for oxygen and glucose lead to a cascade of deleterious effects that cannot be addressed by the delivery of thrombolytics alone. In order to provide more effective neuroprotection, our lab has previously implemented multiple therapeutic modalities using the intraluminal filament rodent model of middle cerebral artery occlusion (MCAO) (Longa et al., 1989). In the present study, in order to enhance the translational potential of these therapeutic modalities, we used a more clinically

*Corresponding authors. Address: Department of Neurological Surgery, Wayne State University School of Medicine, 550 E Canfield, Detroit, MI 48201, USA. Tel: +1-(313)-577-0038; fax: +1-(313)-993-9269 (Y. Ding). China-America Institute of Neuroscience, Luhe Hospital, Capital Medical University, Beijing, China (Z. Ji).

E-mail addresses: jizhili@medmail.com.cn (Z. Ji), yding@med.wayne.edu (Y. Ding).

† Equal contribution.

Abbreviations: EtOH, ethanol; Glut1, glucose transporter 1; Glut3, glucose transporter 3; LDH, lactate dehydrogenase; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; NBO, normobaric oxygen; PFK-1, phosphofructokinase1; rCBF, regional cerebral blood flow; ROS, reactive oxygen species; rt-PA, recombinant tissue plasminogen activator; TH, therapeutic hypothermia.

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relevant model whereby a thrombolytic agent was used to establish reperfusion.

In acute stroke, hyperglycolysis in ischemic regions is initiated from the lack of sufficient oxygen for oxidative phosphorylation (Sako et al., 1985; Yao et al., 1995; Tohyama et al., 1998). Hyperglycolysis leads to increased expression of the brain's main glucose transporters 1 and 3 (Glut1 and Glut3) (Vannucci et al., 1998). In response to hypoxia, the rate limiting enzyme phosphofructokinase1 (PFK-1) is not only activated due to the increased concentration of ADP, AMP, and Pi, but is also upregulated at the transcriptional level, thus leading to hyperglycolysis (Vannucci et al., 2005; Lenzen, 2014). Additionally, a rise of lactate dehydrogenase (LDH) concentration in response to the high glycolytic flux causes lactic acidosis, which is detrimental to cell survival in the ischemic penumbra (Li et al., 2013). During reperfusion, the increased availability of oxygen and glucose causes further damage due to an overproduction of reactive oxygen species (ROS) (Kochanski et al., 2013).

Normobaric oxygenation (NBO) is a simple and logical procedure in treating ischemic stress, However, there is no convincing data to confirm that NBO monotherapy brings about significant benefits to patients after acute ischemic stroke (Bennett et al., 2015). Failure of NBO in clinical trials has not been clear. One reason for these failures might be attributable to hyperglycolysis. Oxygen supplementation in mild to moderate stroke patients may increase mortality because of both reactive oxygen species and xanthine oxidase during reperfusion following ischemia (Rønning and Guldvog, 1999). Oxygen could result in an increase in reactive oxygen species since mitochondrial respiration is impaired after ischemia. Additionally, hyperoxia might hamper cerebral perfusion due to vasoconstriction and mild hyperoxia is associated with a decrease in perfusion of gray matter (Rusyniak et al., 2003; Cornet et al., 2013). In previous stroke treatment studies investigating caffeinol (EtOH + caffeine), low-dose EtOH (0.2–0.65 g/kg, producing blood alcohol content (BAC) of <46 mg/dL) did not demonstrate any neuroprotection when caffeine was omitted (Aronowski et al., 2003). These studies indicate a dose-dependent neuroprotective effect of EtOH, and suggest that the levels of brain catabolism suppressed by low-dose EtOH were not sufficient to protect the brain from ischemia/reperfusion injury.

In rats subjected to a two hour MCAO, we previously demonstrated that a dose of 1.5 g/kg EtOH promotes cell survival, inhibits hyperglycolysis, and preserves neurobehavioral function (Wang et al., 2012; Kochanski et al., 2013). Additionally, we found a synergistic effect when EtOH was combined with NBO administration (Geng et al., 2013b). It is important to note that both of these substances can readily diffuse across the blood brain barrier (BBB). Another established intervention administered either immediately or sometime after reperfusion, is therapeutic hypothermia (TH) which has been shown to blunt several apoptotic triggers, leading to widespread neuronal survival (Olsen et al., 2003; Fingas et al., 2007; van der Worp et al., 2007). While the exact therapeutic mechanism of TH has yet to be elucidated (Goossens and Hachimi-Idrissi, 2014), it may offer a multifaceted protection by reducing intracellular acidosis (Busto et al., 1989), lowering the metabolic rate (Lanier, 1995), and reducing the levels of glycolytic intermediates (Erecinska et al., 2003).

Here we use the new thromboembolic cerebral ischemia model, which mimics events in clinical settings. We have addressed the effect of low NBO concentration (60% but not 95%), alone or in combination with EtOH or TH for neuroprotection. We also demonstrate that EtOH and TH may confer neuroprotection through similar metabolic pathways and EtOH could substitute TH as an alternative therapy for ischemic stroke.

EXPERIMENTAL PROCEDURES

The experimental design and procedures used were in accordance with the National Institute of Health (USA) and approved by the Animal Experimental Committee of Xuanwu Hospital at Capital Medical University. The procedures and data analyses were carried out in a blinded and randomized manner. A total of 138 Sprague–Dawley rats (Beijing Vital River Laboratory Animal Co.) were divided before surgery into a sham group (undergoing the entire surgical procedure except for embolization, $n = 12$) and 7 stroke groups with different treatments. The seven stroke groups included: (1) normothermia and an injection of saline in place of the studied drugs ($n = 18$), (2) rt-PA treatment ($n = 18$), (3) rt-PA + 60% NBO ($n = 18$), (4) rt-PA + TH (33 °C) ($n = 18$), (5) rt-PA + EtOH (1.0 g/kg) ($n = 18$), (6) rt-PA + NBO + TH ($n = 18$), and (7) rt-PA + NBO + EtOH ($n = 18$). Each group was divided into two subgroups for data analyses at 3 and 24 h after the initiation of rt-PA treatment.

Surgical preparation

Sprague–Dawley rats weighing 300–350 g were anesthetized with 2% enflurane. After intubation, the animals were maintained with 1.5% enflurane in 70% nitrous oxide and 30% oxygen through mechanical ventilation. Body temperature was monitored during the procedure with a rectal thermometer and kept at 37 ± 0.5 °C using a feedback-controlled heating blanket. Blood gases and pressure were monitored via the right femoral artery which was cannulated with a PE-50 catheter.

Focal cerebral ischemia

The stroke model was developed previously by Zhang et al. (2015). Briefly, blood was drawn from a femoral artery into 20 cm of PE-50 tubing and incubated initially for two hours in a 37 °C water bath, followed by 22 h in a 4 °C refrigerator. After the above treatment, a section of the clot was cut and transferred to a 20-cm PE-10 tube before flushing it into a petri dish containing sterile saline for five minutes. Repeated rinsing removed most of the red blood cells and created a white embolus, thus